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EXPERIMENTAL THERAPY OF ROCKY MOUNTAIN SPOTTED FEVER.*

THE PREVENTIVE AND CURATIVE ACTION OF A SERUM FOR SPOTTED FEVER, AND THE INEFFICIENCY OF SODIUM CACODYLATE AS A CURATIVE AGENT FOR THIS DISEASE IN GUINEA-PIGS.

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In a preliminary note on the "Production and Concentration of a Serum for Rocky Mountain Spotted Fever"¹ we reported the preparation of a serum for Rocky Mountain spotted fever and its potency, determined by tests on guinea-pigs. Additional experiments have confirmed our previous results and have shown that the serum has a limited curative effect on guinea-pigs inoculated with spotted fever virus, if given during early stages of the disease. A series of experiments upon the efficacy of sodium cacodylate as a curative agent has also been completed, the results of which are reported in this paper.

SPOTTED FEVER SERUM.²

In the spring of 1907 Ricketts and one of us (H.), while working on spotted fever problems in Missoula, Mont., inoculated two horses with spotted fever virus. One of the horses was inoculated subcutaneously with 65 c.c. obtained from infected guinea-pigs, the other horse with 80 c.c. from a monkey. The first horse had a temperature of 105.6° on the third day after inoculation. The temperature fell rapidly and no tests were made of the infectiousness of the blood or of the protective power of the serum. Another injection of 60 c.c. blood from a spotted fever patient and an infected monkey gave no reaction. The second horse did not

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¹ *Jour. Am. M. Ass.*, 1911, 57, p. 198.

² Early experiments with horses, carried on by the late Dr. Ricketts in conjunction with us, have never been published in full. Brief reference is made to the work with two horses in Missoula in 1907 in *Jour. Am. M. Ass.*, 1907, 49, p. 24. Other results of his work mentioned in this paper are taken from Dr. Ricketts' original notes.

react. The following winter (1908) we inoculated two horses. The first one of these received 110 c.c. guinea-pig virus intraperitoneally. Some blood was drawn before injection to test the natural protective power of horse's blood. The results were negative. The maximum temperature was reached on the third day (103.6°). Several tests of the infectivity of the blood for guinea-pigs during the course of the fever were made. A positive result was obtained only from the blood obtained on the day of highest temperature and the result confirmed by passage from the first guinea-pig to a second one. Both had typical spotted fever determined by the temperature curve, external lesions, and autopsies. The protective power of serum drawn after subsidence of fever was 1 c.c. The second horse inoculated did not react. The first horse was bled to death and the serum put up in vials containing about 20 c.c. each and sent to the Bitterroot Valley in Montana for use. No systematic reports were received of its value in the treatment of the disease. Two or three injections with this serum caused severe rashes, which vanished after a day or two, and probably were due to the well-known effect of fresh horse serum.

During the same year (1908) Ricketts, assisted by one of us (M.), inoculated two horses. One received 80 c.c. guinea-pig virus, the other 85 c.c. guinea-pig virus and an emulsion of the livers and spleens of the same guinea-pigs. Results in both cases were negative.

We resumed this work in the early spring of 1911 with financial assistance of the counties of Ravalli and Missoula in western Montana. Two horses were purchased and injected with virus. A third horse, also injected, did not respond. The first horse received 220 c.c. guinea-pig virus. The virus was obtained by injecting guinea-pigs and bleeding them to death on the second day of the fever. The defibrinated blood was injected. Previous experiments were not extensive enough to show preference for any method of injection. For this reason we concluded to divide the virus into two equal parts and inject one part into the jugular vein in the neck, and the other part subcutaneously back of the shoulder. About three weeks later this horse received an injection of 460 c.c. virus, also by intravenous and subcutaneous

injections. The second horse received but one injection of 250 c.c. guinea-pig virus.

The temperatures were taken twice daily and are recorded in Table I. The maximum temperature of horse 1 was 103.5° on the fourth day after injection, of horse 2 on the third day (103.4°). After horse 1 had received the second injection no appreciable fever developed, excepting for an hour or two shortly after injection. This was probably due to anaphylactic shock, as the horse was covered with a profuse perspiration and trembled violently.

TABLE I.
TEMPERATURES OF HORSES INJECTED WITH SPOTTED FEVER VIRUS.

HORSE	TEMPERATURES AFTER DAYS											
	0		1		2		3		4		5	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1.....	102.6	101.2	99.9	99.8	101.6	100.6	102.0	102.8	103.5	102.1	102.5	
2.....	100.8	98.9	98.8	101.0	101.0	102.4	103.4	103.4	102.0	101.0	100.8	
I. After 2d injection....	100.8	100.5	101.8	100.8	100.2	101.5	101.2	101.2	101.2	101.0	100.8	

TABLE I.—Continued.

HORSE	TEMPERATURES AFTER DAYS											
	6		7		8		9		10		11	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1.....	101.8	103.0	101.8	102.4	101.0	101.0	99.6	99.8	99.6	99.6	99.4	99.8
2.....	102.0	99.4	100.4	100.5	99.2	99.8	98.8	99.2	99.2	99.4	99.2	99.4
I. After 2d injection...	100.2	100.5	99.8	99.6	99.8	99.8	99.6	98.8	98.8	99.2	99.6	99.8

Both horses were bled every day during the period of temperature and the defibrinated blood injected into guinea-pigs in doses of 1 c.c. and 5 c.c. A positive result was obtained only the day of the highest temperature in horse 1. The result was confirmed by passage from the first guinea-pig into a second one. Curiously the 1 c.c. injection only was positive. The 5 c.c. injection showed no result. This observation was made by Ricketts also and he explained the phenomenon on the ground of the presence of immune

bodies in horse's blood. The mild course of the fever in the horse would tend to confirm this theory. Through oversight no blood was drawn from horse 2 on the day of highest temperature, and all tests on guinea-pigs were negative.

After the fever had subsided blood was drawn every second day and the serum tested for protective power. At first the results were negative, but gradually protection seemed to develop and reached the highest efficiency after 12 days. These tests were made by injecting 1 c.c. virus in all cases and varying amounts of serum. The virus and serum were injected separately into the peritoneal cavity. We used guinea-pigs weighing approximately 250 gms. It is true that according to Ricketts' statements 0.1 c.c. virus is always infectious, and 0.05 c.c. in most cases, and 0.01 c.c. in many cases. However, we aimed to make the tests as rigorous as possible and used 1 c.c. The virus and serum were never mixed before injection, to eliminate a possible reduction of virulence by the action of the serum. As controls we injected in all experiments reported a guinea-pig with virus only and one with virus and 5 c.c. normal horse serum. The results were considered positive if the guinea-pig survived without having shown marked rise in temperature. If the guinea-pigs died the diagnosis of spotted fever was based on temperature curves, external lesions, and the findings after autopsies.

In order not to burden the tables with too many figures we have recorded only one typical temperature curve of guinea-pigs used for controls and only one where normal horse serum was used. We have tabulated these in Table 2 together with some selected temperature protocols, representing the limits of potency of the sera. The serum was diluted with physiological salt solution to 4 c.c. in one series of experiments. For some unexplained reason the guinea-pigs endured injections of undiluted serum better than of diluted serum. Those injected with diluted serum seemed to succumb to the influence in many instances, while those injected with undiluted serum showed no untoward effect.

The table shows that the native serum of horse 1 drawn after 12 days protected in doses of 5 c.c., but not if only 1 c.c. was given. We did not determine the amount between 1 and 5 c.c. which

TABLE 2.
TEST OF POTENCY OF SPOTTED FEVER SERA AGAINST I. C.C. VIRUS.

KIND OF SERUM	AMOUNT	NO. OF GUINEA-PIGS	TEMPERATURE AFTER DAYS												REMARKS				
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Horse 1. Serum drawn 12 days after temperature subsided	0.1 c.c.	1	102.8	102.8	102.4	102.6	102.2	104.6	106.0	106.0	106.2	106.0	103.8	103.4	103.0	102.6	102.4	102.5	No protection, typical course of spotted fever
	0.5 c.c.	2	102.6	102.6	102.6	103.0	103.6	102.6	105.4	106.2	106.0	105.2	105.4	105.4	105.4	105.2	102.6	102.5	Died of spotted fever after 12 days
	1.0 c.c.	3	102.8	103.4	103.6	104.6	104.8	105.2	106.0	106.2	106.0	105.4	105.4	105.4	105.4	105.2	102.6	102.5	Died of spotted fever after 12 days
	5.0 c.c.	4	102.0	102.6	102.8	103.0	103.0	103.6	103.0	103.0	102.6	102.6	102.6	102.8	102.6	102.8	102.6	102.6	Protected
Control, virus only	5	102.8	102.8	103.6	104.6	105.2	106.2	106.0	105.2	105.2	105.2	105.2	105.2	105.2	105.2	105.2	105.2	Died of spotted fever after 9 days
Virus and normal horse serum....	5.0 c.c.	6	102.2	102.4	102.4	103.2	103.2	105.0	105.2	105.8	104.0	106.2	105.4	106.2	105.8	104.0	102.6	102.5	Died of spotted fever after 10 days
Horse 2. Serum drawn 2 days after 2d injection	0.125 c.c.	7	102.4	102.0	102.0	103.2	102.8	102.2	103.6	103.8	104.0	105.2	106.0	105.0	104.0	103.0	104.0	103.0	Fever delayed, died of spotted fever after 14 days
	0.25 c.c.	8	102.6	103.2	102.2	103.2	102.8	102.6	103.0	103.2	103.6	104.8	105.0	105.2	105.2	104.0	102.2	101.8	Mild course of fever, recovered
	0.5 c.c.	9	102.8	103.0	102.2	103.0	102.8	102.4	103.2	102.8	102.6	102.4	102.0	102.8	102.0	103.0	102.8	102.0	Complete protection
Horse 2. Serum drawn 12 days after 2d injection	0.125 c.c.	10	103.0	102.2	103.2	103.0	103.2	103.6	102.6	103.0	103.6	104.2	105.0	105.2	105.2	105.0	103.0	102.8	Mild fever, probable protection
	0.5 c.c.	11	102.8	103.0	102.2	103.0	102.8	102.4	103.2	102.8	102.0	103.0	102.6	103.8	103.0	104.0	102.2	102.0	Complete protection
	0.75 c.c.	12	102.6	102.6	102.0	102.4	102.4	102.0	103.2	103.0	102.6	102.4	104.0	103.6	102.6	103.8	102.6	102.6	Complete protection

would protect. The serum from horse 2 protected if 0.5 c.c. were given. If 0.25 c.c. were given the fever ran a mild course and the guinea-pig recovered. The serum of horse 1 drawn after the second injection protected definitely if 0.5 c.c. were given, while doses of 0.25 and 0.125 c.c. seemed to cause the fever to take a mild course.

Since antidiphtheric serum has been concentrated successfully during recent years we thought that possibly spotted fever serum might be concentrated by the same method. The experiment was successful, as is shown in Table 3. The serum from horse 1 after the second injection of virus was concentrated and then protected if 0.05 c.c. were given. The concentrated serum of horse 2 protected in doses of 0.01.

The favorable results of concentrating the serum show that the antibodies to spotted fever in horse's blood are united with the pseudo-globulin fraction, the same as in diphtheria and tetanus antitoxin. The decided gain in potency resulting from the second injection given to horse 1, coupled with the possibility of increasing the potency by concentration, seem to indicate that by giving frequent injections of virus according to a similar plan now carried on in the preparation of diphtheria antitoxin, highly potent sera may be obtained. We expect to test this possibility as soon as opportunity offers.

The concentrated serum of horse 2 was put up in vials containing about 25 c.c. each and sent to Missoula for use during the season of spotted fever. The serum was, of course, sterilized by passing through a Berkefeld filter, and a safety test made by injecting 5 c.c. into the peritoneal cavity of a guinea-pig. It seems that not sufficient interest was taken in trying this serum, so that its efficiency for human beings remains problematical. The eradication of spotted fever in the Bitter Root Valley is a matter of vital importance, not only locally, but also because of the likelihood of the fever spreading throughout the neighboring states as the population increases. By systematic attempts at eradicating the ticks and the lower animals serving as hosts for ticks much may be accomplished. These methods, however, will take considerable time before results can be expected, and it would seem that a serum of prophylactic and possibly curative value might afford immediate

TABLE 3.
TESTS OF POTENCY OF CONCENTRATED SPOTTED FEVER SERA AGAINST I.C.C. VIRUS.

KIND OF SERUM	AMOUNT	No. OF GUINEA-PIGS	TEMPERATURE AFTER DAYS										REMARKS						
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Horse 1. Concentrated serum after 2d injection	0.01 c.c.	13	102.6	102.6	102.6	102.6	103.0	103.6	104.6	105.0	105.0	105.0	104.2	104.6	104.4	104.6	104.0	102.6	
	0.03 c.c.	14	103.4	102.4	102.4	102.4	102.0	101.2	101.2	101.2	101.2	101.2	101.2	104.6	104.8	104.6	104.0	103.8	102.4
	0.05 c.c.	15	103.0	102.0	102.0	102.0	101.2	103.2	103.2	103.2	103.2	103.2	103.2	103.0	103.2	103.0	102.4	102.6	102.4
Horse 2. Concentrated serum.....	0.01 c.c.	16	102.6	103.0	102.8	102.4	102.1	102.2	102.8	103.0	103.4	103.0	102.6	102.0	102.2	102.0	102.2	102.0	102.2
	Concentrated serum after 12 mos..	0.08 c.c.	22	103.0	102.6	102.6	102.8	103.6	105.0	104.3	104.0	103.8	103.6	102.4	102.6	102.6	102.6	102.6	102.6

relief. Systematic administration as prophylactic in cases of tick bites in infected regions and as curative agent during early stages of the disease is the course indicated to determine this question.

In order to confirm all potency tests the guinea-pigs surviving were given immunity tests by injecting 1 c.c. virus. The results appear in Table 4. All but number 4 did not take the disease and were therefore immune. Whether this is passive or active immunity is difficult to decide. We are inclined to think it is passive immunity. At any rate immunity lasted for at least four weeks, as is evident from the fact that a second immunity test in guinea-pigs 8, 9, 10, 11, and 12 was positive, inasmuch as the guinea-pigs did not take the disease from a second injection of 1 c.c. virus. In guinea-pig 4 the immunity was probably of short duration, since it ran a typical, although prolonged, course of spotted fever and died on the 12th day.

To determine the amount of deterioration of protective value of concentrated serum from horse 2, a series of tests was made after the lapse of 12 months, during which time it was kept in an ice chest. The former protective dose of 0.01 was reduced to 0.08, and partial protection afforded with 0.05. The same serum protected in subcutaneous injections in a dose of 0.1 c.c. Smaller amounts were not tested.

The next series of experiments were conducted with the object of determining whether the serum can influence the course of the disease, if given at various periods after injection of the virus. We inoculated five guinea-pigs with 1 c.c. virus and then injected 1 c.c. concentrated serum on different days. Guinea-pig 17 received serum on the first day after injection of virus, guinea-pig 18 on the second day, guinea-pig 19 on the third day, guinea-pig 20 on the fourth day, and guinea-pig 21 on the fifth day. The results appear in Table 5. Guinea-pigs 17 and 18 had but slight changes in temperature and recovered completely. The serum evidently took effect before the first appearance of temperature. Guinea-pig 19 had a temperature of 105.2° on the day the serum was given. The temperature was down to 103° the next day, and no important changes took place after that and the animal recovered. Guinea-pigs 20 and 21 received serum after temperature was high for one

TABLE 4.
IMMUNITY TESTS ON GUINEA-PIGS, WHICH SURVIVED IN TABLES 2 AND 3.

No. OF GUINEA- PIG	TEMPERATURE AFTER DAYS															REMARKS
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.....	102.8	102.6	103.0	102.6	103.4	103.2	103.0	102.0	102.6	103.0	102.6	102.6	102.8	103.6	102.6	Immune
4.....	102.6	102.8	103.2	103.0	105.0	105.6	106.0	106.0	105.0	105.4	105.6	105.0	105.2	105.2	105.2	Died of spotted fever after 11 days
8.....	103.6	103.8	104.0	103.0	103.4	103.0	102.8	102.6	102.8	103.0	102.8	103.0	103.0	103.2	103.2	Immune, second immunity test gave the same result
9.....	103.0	103.8	103.8	103.6	103.6	102.8	103.0	103.0	103.4	103.2	103.4	103.2	103.2	103.2	103.2	Immune, second immunity test gave the same result
10.....	103.0	103.6	103.6	103.4	103.8	103.6	103.4	103.0	103.4	103.0	103.4	103.4	103.4	103.4	103.4	Immune, second immunity test gave the same result
11.....	103.2	104.0	105.8	103.6	103.4	103.2	103.4	103.0	103.2	103.0	103.2	103.2	103.2	103.2	103.2	Immune, second immunity test gave the same result
12.....	103.2	104.2	105.8	103.8	103.6	103.6	103.6	103.4	103.4	103.4	104.0	104.6	104.8	103.8	103.8	Immune, second immunity test gave the same result
13.....	103.4	103.0	103.8	103.0	103.6	102.6	102.6	102.8	102.8	102.6	102.6	102.6	102.6	102.4	102.4	Immune, second immunity test gave the same result
14.....	103.6	103.6	103.8	103.5	103.5	103.6	102.6	102.6	102.6	102.6	102.6	102.6	102.6	102.6	102.6	Immune, second immunity test gave the same result
15.....	103.6	102.0	103.0	103.0	103.4	103.2	102.4	103.2	102.4	102.6	102.6	102.6	102.4	102.4	102.4	Immune, second immunity test gave the same result
16.....	102.6	102.6	102.6	102.6	103.0	101.0	101.0	102.0	101.0	101.0	101.0	101.0	101.0	101.0	101.0	Immune, second immunity test gave the same result

TABLE 5.
CURATIVE VALUE OF CONCENTRATED SERUM FROM HORSE 1, AFTER SECOND INJECTION.

DAYS AFTER WHICH SERUM WAS INJECTED	No. OF GUINEA- PIG	TEMPERATURE AFTER DAYS										REMARKS					
		0	1	2	3	4	5	6	7	8	9						
1.....	17	102.6	103.2	102.0	101.0	100.0	103.4	103.2	103.4	103.0	103.0	103.0	102.6	102.6	102.6	Complete protection	
2.....	18	102.4	102.4	102.5	103.0	104.0	103.9	103.0	104.0	103.8	103.0	103.0	102.1	102.2	102.2	Complete protection	
3.....	19	102.6	102.6	102.4	105.2	105.2	105.2	105.2	105.2	105.2	105.2	105.2	104.1	103.9	102.5	102.5	Complete protection
4.....	20	102.0	103.0	103.0	104.0	104.0	104.0	104.0	105.2	105.2	105.2	105.2	104.2	104.2	104.2	Died of spotted fever on 11th day	
5.....	21	102.4	102.8	103.2	104.2	105.0	105.0	105.6	106.0	105.8	105.2	105.2	105.2	105.2	105.2	Died of spotted fever on 9th day	

and two days respectively. Both animals died of spotted fever. Control animals were inoculated as usual. These experiments seem to show that the serum may have value as a prophylactic measure and may even have curative effect. It must, however, be given early in the disease. After temperature has persisted for some time the serum seems to be ineffective. One c.c. in a guinea-pig corresponds to about 300 c.c. in a man of 150 pounds weight. The serum should be given in large doses and the injection repeated at frequent intervals. No harm can result from large doses, as has been demonstrated many times by the administration of several 100,000 units of diphtheria antitoxin. We did not determine whether smaller amounts than 1 c.c. serum have prophylactic effects on guinea-pigs.

We intend to take up experiments with the preparation and effect of serum from immunized horses when opportunity offers. Our success in increasing the potency materially by a second injection encourages the belief that repeated injections of virus will produce a powerful serum, the potency of which can be increased materially by concentration.

EXPERIMENTS WITH CACODYLATE OF SODIUM.

Recently claim has been made that sodium cacodylate is effective in the cure of spotted fever. Ricketts and Matthews some time ago carried on preliminary experiments, which were not published because the results were not conclusive.¹ We therefore undertook a series of experiments with different amounts of this drug. Ricketts and Matthews determined that 8 mgm. was the largest amount that guinea-pigs will stand without untoward effect. We confined our tests to doses varying from 2 mgm. to 8 mgm. The injections were given subcutaneously. Twenty-six guinea-pigs were inoculated with 1 c.c. virus each. On the third day six guinea-pigs received 2 mgm. sodium cacodylate dissolved in sterile water. Another set of six guinea-pigs received 4 mgm., a third set 6 mgm., and a fourth set 8 mgm. The remaining two guinea-pigs were reserved as controls. The injection of cacodylate of sodium was repeated every day until death ensued. All these guinea-pigs, including the controls, died of spotted fever in seven to nine days.

¹ They also tested the efficiency of atoxyl, spirarzyl, and quinine sulphate with negative results. We confined our experiments, therefore, to sodium cacodylate.

No difference could be detected between the guinea-pigs which had been treated with cacodylate of sodium and the two controls. The temperature curve was typical of spotted fever in all cases and the diagnosis was confirmed by autopsies. We feel justified in the conclusion that sodium cacodylate has no effect on the course of spotted fever in guinea-pigs.

CONCLUSIONS.

1. Horses are susceptible to spotted fever if the virus of guinea-pigs is injected subcutaneously and intravenously. The fever usually takes a mild course and the temperature is not exceedingly high. Normal temperature appears again after seven to nine days. It would be interesting to allow infected ticks to bite horses and determine whether the disease can be communicated that way.
2. The serum from horses recovered from spotted fever has protective value. The potency is largest after about 12 days from the time of the reappearance of normal temperature.
3. Repeated injection of spotted fever virus increases the potency of the serum materially, but does not produce a second attack of spotted fever.
4. The serum can be concentrated by the method practiced in concentrating diphtheria antitoxin. The gain in potency may be 10 times the original value.
5. The pseudoglobulin fraction of blood serum contains the bulk of antibodies in immunized horses.
6. Assuming 0.1 c.c. of guinea-pig virus to be the smallest amount which will invariably produce spotted fever in guinea-pigs, 1 c.c. of a serum was protective against 1,000 doses.
7. Guinea-pigs injected with spotted fever virus and immune horse serum separately into the peritoneal cavity acquire an immunity lasting for at least four weeks.
8. One c.c. immune horse serum protects guinea-pigs injected with spotted fever virus up to, and including, the first day of high temperature. If serum is given later there is no protection.
9. Treatment of guinea-pigs, injected with spotted fever virus, with sodium cacodylate and repeated every day, commencing with the first appearance of temperature, has no effect on the course of the disease.